

## Ultrastructural changes during megasporogenesis in *Epipactis* (*Orchidaceae*)

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### Abstract

Plastids were temporarily localized within the micropylar portion of the early first prophase *Epipactis* meiocyte. Some of these plastids were observed in close proximity to the nuclear envelope. With the exception of this short period, plastids were distributed randomly in the meiocyte. During late prophase, starch-containing plastids become cup-shaped and depleted of starch. Plastids were found within both dyad cells and all cells of the tetrad. Elongated segments of ER cisternae in various configurations were present. The chalazal wall of the prophase meiocyte differed from other walls in the presence of the ingrowths and plasmodesmata. The micropylar portion of the nuclear envelope at some stages of the I prophase seemed to be devoid of pores whereas the chalazal part contained numerous pores. These structural characters reflect a polar differentiation of the meiocyte along a micropylar-chalazal axis.

### INTRODUCTION

The ultrastructure of the certain stages of megasporogenesis in *Orchis* has been described (Israel, Sagawa, 1964; Coccuci, 1969). Previously, Rodkiewicz and Stobiecka (1978) reported that starch grains and plastids were randomly distributed within premeiotic and early leptoten cells but that they accumulated with time within the micropylar portion of the meiocyte and that at late pachytene, starch grains were again distributed throughout the meiocyte. Bednara et al. (1977) and Rodkiewicz and Bednara (1976) detailed the distribution of plasmodesmata and wall ingrowths in the chalazal wall of the meiocyte during megasporogenesis in *Epipactis*. They observed that the chalazal wall resembled a transfer wall in that it contained ingrowths. In addition, plasmodesmata were seen to transverse the

chalazal part of the meiocyte cell wall. Bednara (1978) suggested that the differentiated chalazal wall functions in the polarization of the meiocyte.

## MATERIAL AND METHODS

Ovules of *Epipactis palustris* (Mill.) were fixed in 4% glutaraldehyde and postfixed in 2% osmium tetroxide or in 1% potassium permanganate. The material was embedded in Epon and ultrathin sections were stained with uranyl acetate and lead citrate (Reynolds, 1963).

## RESULTS

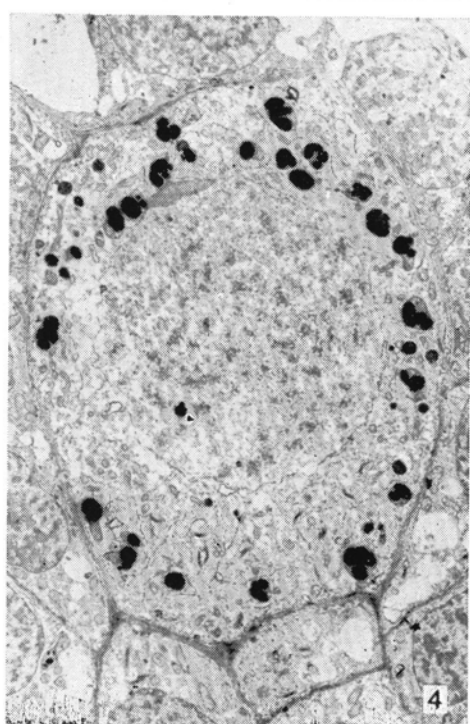
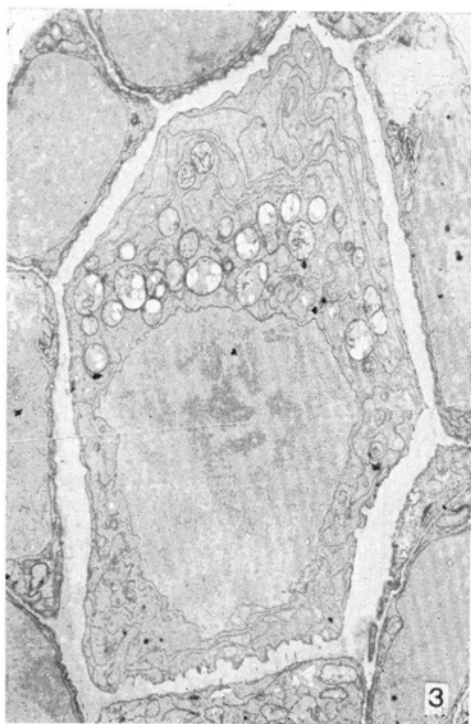
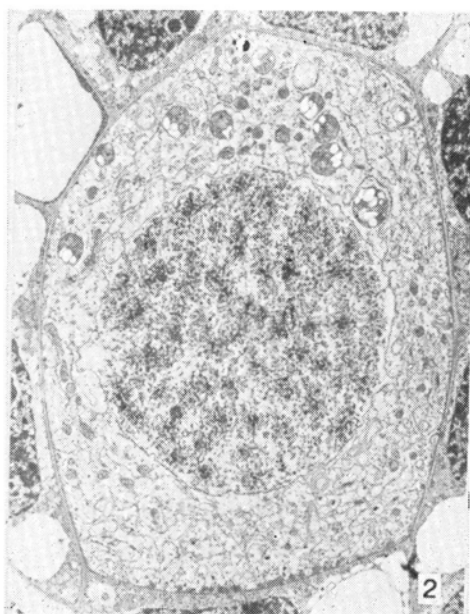
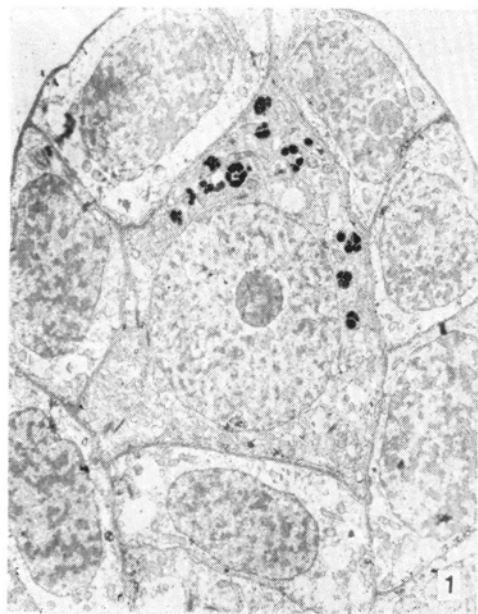
The present paper reports additional observations on the distribution of plastids, ER, nuclear pores and wall ingrowths within the meiotic cell of *Epipactis palustris* ovules.

### Plastids

Figs 1-3 show the distribution of subcellular organelles within the *Epipactis* megasporocyte in the early meiotic prophase. Plastids with starch grains, which stained with the Thiéry (1967) reaction (Fig. 1) were localized within the micropylar portion of the megasporocyte. This localization was seen following either glutaraldehyde-osmium or  $\text{KMnO}_4$  fixation (Figs 2, 3). The plastids which occupied the micropylar region of the megasporocyte, were observed in close proximity to the nuclear envelope (Fig. 5). At a later stage of prophase, starch containing plastids encircled the meiocyte nucleus (Fig. 4). With time they became cup-shaped and depleted of starch (Figs 6, 7). Plastids were found within both cells of a dyad (Fig. 8) and all cells of the tetrad.

### Endoplasmic reticulum

The ER underwent distinct ultrastructural changes during megasporogenesis. Elongated segments of ER cisternae were present within the prophase meiocyte. Some cisternae were in parallel array at earlier prophase (Fig. 12) as concentric lamellae at anaphase I (Figs 7, 13). A large number of randomly distributed elongated ER cisternae were noted within the cells of a dyad, such cisternae were rarely seen within the developing functional megaspore.



Figs 1-3. *Epipactis palustris* megasporocyte in early prophase I

Fig. 1. Plastids with starch grains, after Thiéry reaction, gathered in micropylar part of the cell. 2100 $\times$ .

Fig. 2. Plastids in micropylar part after glutaraldehyde fixation. 3100 $\times$ .

Fig. 3. Plastids in micropylar part after glutaraldehyde fixation and  $\text{KMnO}_4$  postfixation. 3100 $\times$ .

Fig. 4. Plastids with starch, after Thiéry reaction, encircling meiocyte nucleus at a later stage of prophase I. 3100 $\times$ .

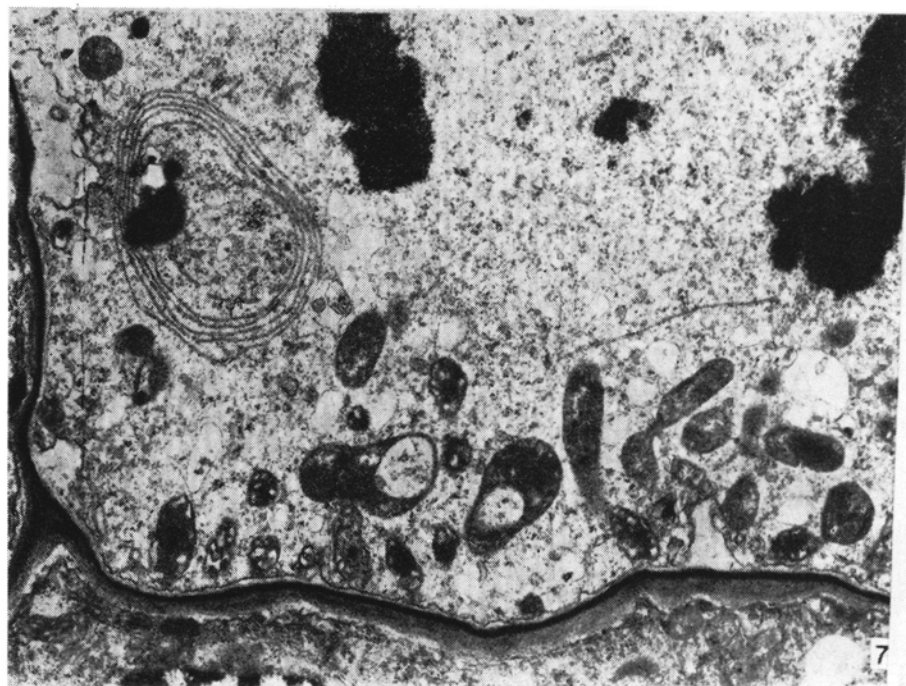
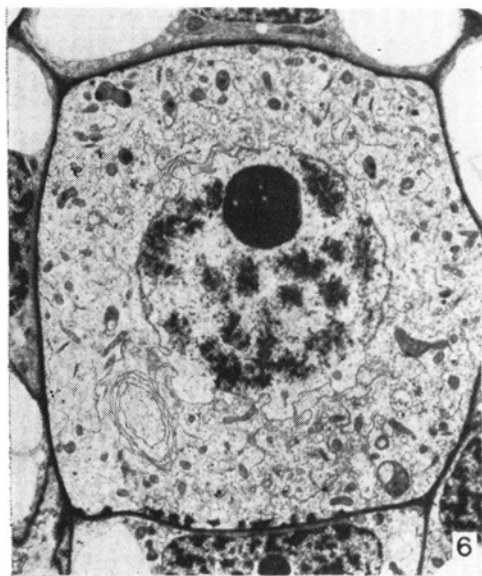
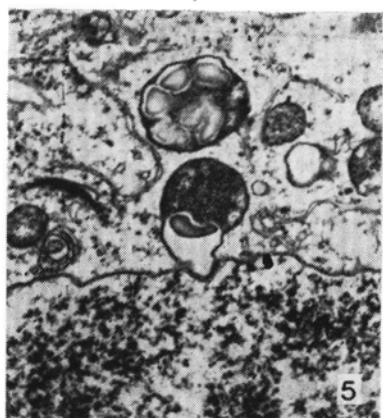


Fig. 5. Plastid from micropylar group in close contact with nuclear envelope. 9000 $\times$

Fig. 6. Meiocyte prophase I with scattered plastids and concentric ER cisternae, chalazal wall ingrowths. 3000 $\times$ .

Fig. 7. Anaphase I, concentric ER cisternae, cup-formed plastids depleted of starch. 9000 $\times$ .

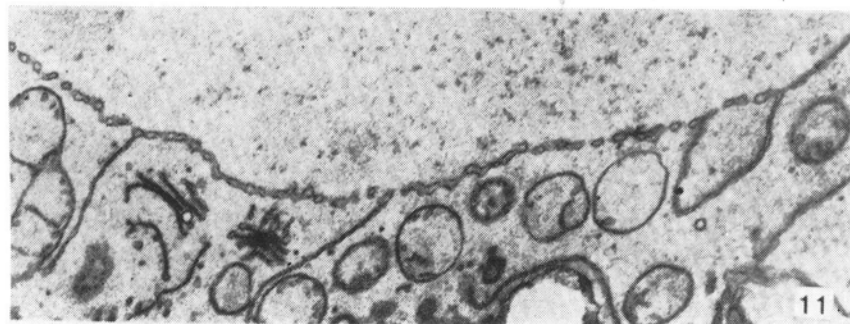
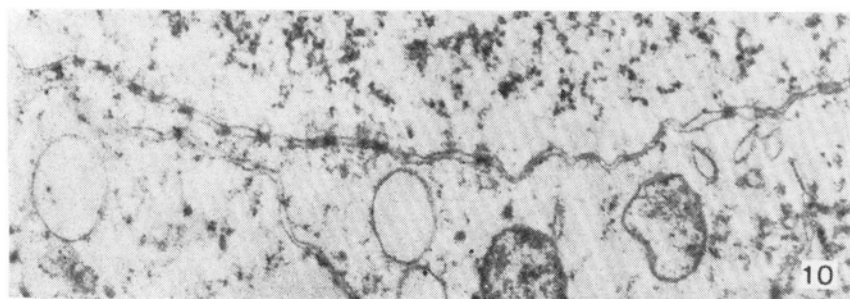
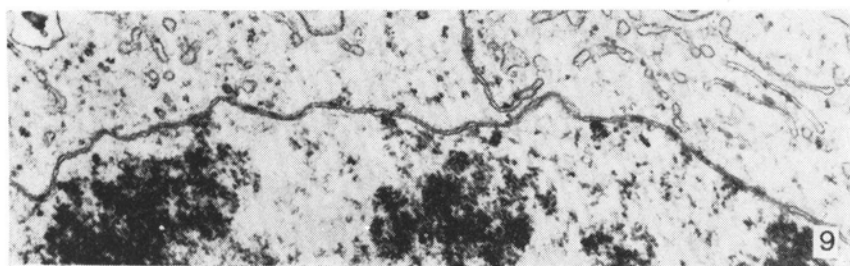
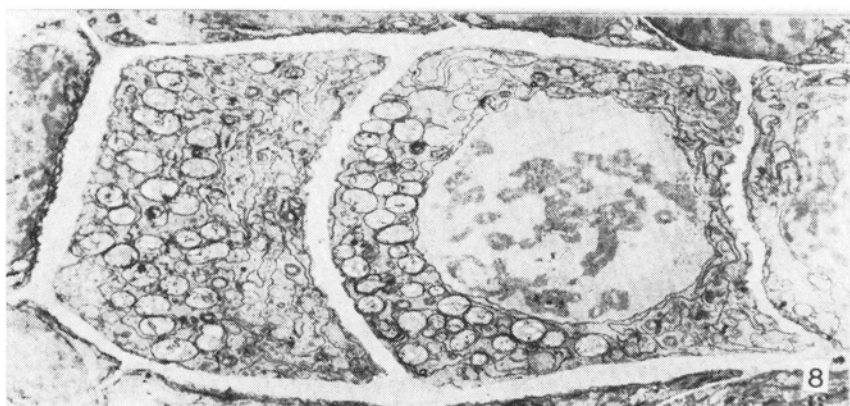


Fig. 8. Plastids in both cells of dyad post-fixed with  $\text{KMnO}_4$ . 4000 $\times$   
 Fig. 9. Micropylar part of nuclear envelope of early prophase I meiocyte. 18000 $\times$   
 Fig. 10. Chalazal part of nuclear envelope showing pores following glutaraldehyde-osmium fixation in the same stage meiocyte. 18000 $\times$   
 Fig. 11. Chalazal part of nuclear envelope after  $\text{KMnO}_4$  fixation. 18000 $\times$

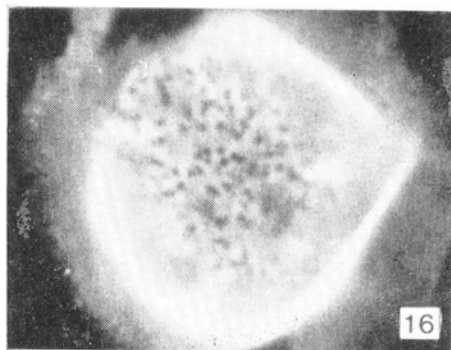
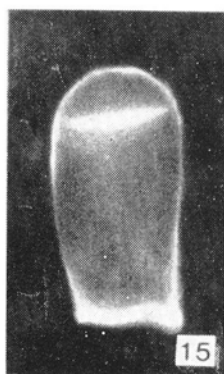
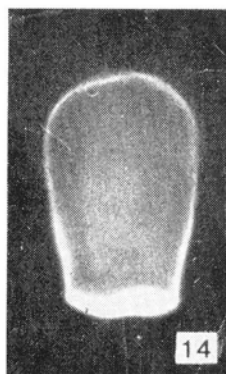
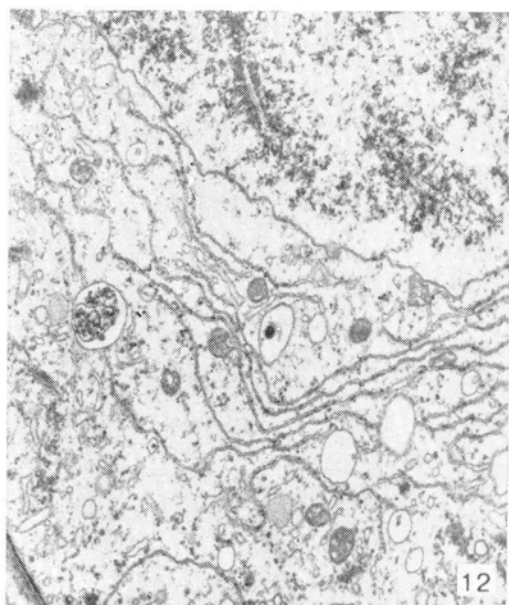


Fig. 12. Parallel ER cisternae in synaptonemal meiocyte. 8000 $\times$ .

Fig. 13. Anaphase I, stains of ER cisternae. 9009 $\times$

Fig. 14. Late prophase I meiocyte, fluorescent wall after staining with aniline blue. 600 $\times$

Fig. 15. Dyad — fluorescent wall after staining with aniline blue. 600 $\times$

Fig. 16. "Sieve-like" distribution of fluorescent material in meiocyte chalazal wall after aniline blue staining. 2000 $\times$



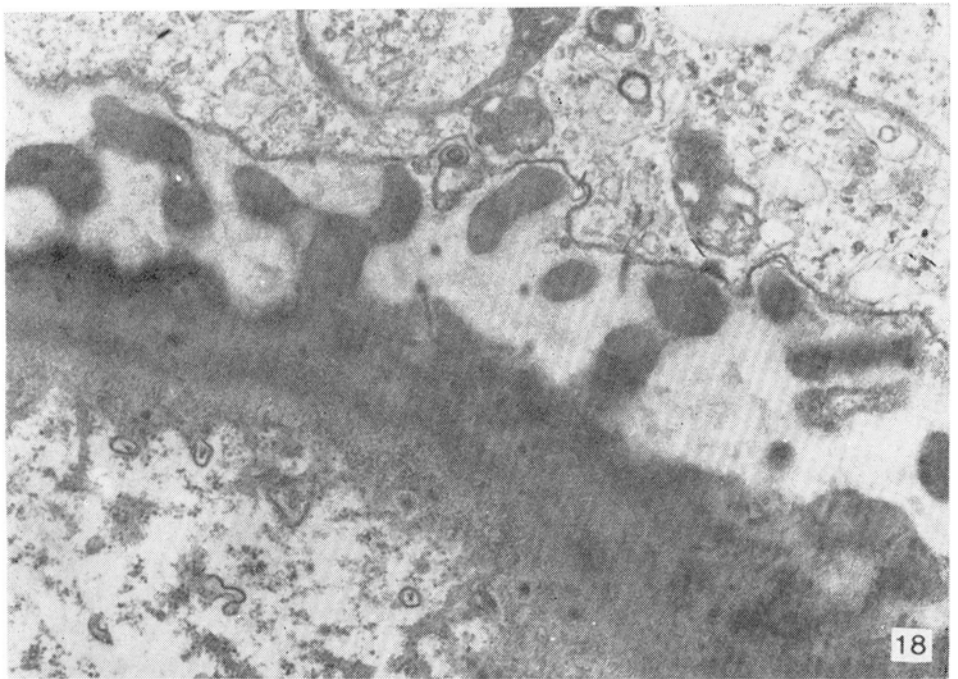
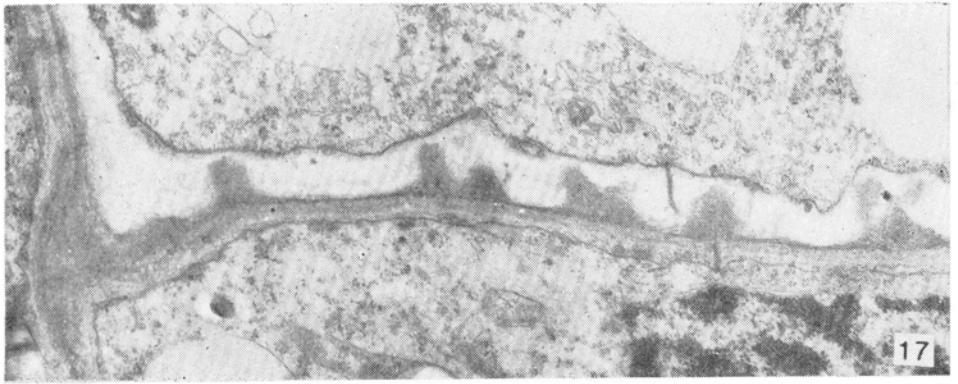


Fig. 17. Chalazal wall in meiocyte showing plasmodesmata and ingrowths projecting into electron luscent area. 12000 $\times$

Fig. 18. Oblique section of the wall shown in Fig. 17. Ingrowths in electron luscent layer, cross section of plasmodesmata in dark layer and transverse in light area. 12000 $\times$

### Nuclear envelope

Comparison of Figs 9 and 10 demonstrates that there is a structural difference between the micropylar and chalazal portions of the nuclear envelope during some period of meiotic prophase I. Whereas the micropylar portion of the envelope seemed to be devoid of pores, the chalazal part contained numerous pores. Following glutaraldehyde-osmium fixation, pores were filled with an electron dense material which was absent from permanganate fixed cells (Fig. 11).

### Wall ingrowths

The chalazal wall of the early prophase meiocyte and functional megaspore differed from other walls by the presence of small ingrowths which were apparent in either longitudinal (Fig. 17) and oblique (Fig. 18) sections. When the thick callose layer was deposited within the late prophase meiocyte wall (Fig. 14) and dyad (Fig. 15), a chalazal portion of it appeared to be perforated by wall ingrowths. The chalazal wall of the meiocyte displayed a sieve-like distribution of fluorescent material upon staining with aniline blue (Fig. 16).

### DISCUSSION

The distribution of plastids in the meiocyte during the first meiotic prophase changed from random to exclusively micropylar at late leptotene and again to random at later prophase. At present time there is no obvious explanation for this temporary change in distribution. This phenomenon does not appear to be restricted to megasporogenesis in orchids. For example, a preferential localization of plastids has been reported for the meiocyte of *Equisetum* (Marquette, 1907) and *Marsilia quadrifolia* (Marquette, 1908). Similar observations were made for *Equisetum variegatum* (Lenoir, 1934), but Jungers (1934) was unable to repeat the observation. The possible explanation for this discrepancy is that the time period during which the micropylar localization of plastids occur is only a small fraction of meiotic prophase I. Thus, this preferential localization of plastids could be easily missed.

The structural difference between the micropylar and chalazal portions of the nuclear envelope as well as presence of ingrowths and plasmodesmata in the chalazal cell wall may reflect a polar differentiation of the meiocyte along a micropylar-chalazal axis.

The conversion of elongated segments of ER cisternae within the meiocyte to concentric lamellae and tight stack configurations may indicate that mid-prophase and anaphase I meiocytes reduce some synthetic activity present in the early prophase meiocyte.



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